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Review

Metabolic reprogramming in liver fibrosis

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SUMMARY

Chronic liver diseases, primarily metabolic dysfunction-associated steatotic liver disease (MASLD), harmful use of alcohol, or viral hepatitis, may result in liver fibrosis, cirrhosis, and cancer. Hepatic fibrogenesis is a complex process with interactions between different resident and non-resident heterogeneous liver cell populations, ultimately leading to deposition of extracellular matrix and organ failure. Shifts in cell phenotypes and functions involve pronounced transcriptional and protein synthesis changes that require metabolic adaptations in cellular substrate metabolism, including glucose and lipid metabolism, resembling changes associated with the Warburg effect in cancer cells. Cell activation and metabolic changes are regulated by metabolic stress responses, including the unfolded protein response, endoplasmic reticulum stress, autophagy, ferroptosis, and nuclear receptor signaling. These metabolic adaptations are crucial for inflammatory and fibrogenic activation of macrophages, lymphoid cells, and hepatic stellate cells. Modulation of these pathways, therefore, offers opportunities for novel therapeutic approaches to halt or even reverse liver fibrosis progression.

INTRODUCTION

The liver is a central regulator of metabolism and located at the nexus of portal and systemic blood flow, where it is exposed to nutrients, bacterial products, and other substances crucial for metabolic processes and detoxification within the body. The liver is well known for its regenerative capacity, with the potential to fully recover even after severe acute injury or partial organ removal.[1] Still, all liver injury elicits a wound-healing response, with liver inflammation and the deposition of the extracellular matrix (ECM), even after acute hepatic damage.[2] Chronic liver diseases caused by repeated and long-lasting injury, including exposure to hepatotoxins, insulin resistance-associated metabolic dysfunction, chronic infections, and autoimmune conditions, are characterized by an excessive wound-healing response with excess ECM deposition, called liver fibrosis.[3] Mechanistically, signals from damaged liver cells—hepatocytes and cholangiocytes—cause endothelial activation, immune cell recruitment, and their inflammatory activation and differentiation. Kupffer cells and hepatic monocyte-derived macrophages are attracting particular interest, as recent single-cell RNA sequencing and proteomic studies have uncovered the remarkable heterogeneity and plasticity of hepatic macrophages, with distinct phenotypes being involved in the initiation and perpetuation of injury and fibrogenesis, as well as their resolution.[4] Damage-associated and immune cell-derived signals eventually culminate in the activation of hepatic stellate cells (HSCs)—retinol-storing pericytes of the hepatic sinusoids—that, upon activation, acquire matrix-producing, proliferative, migratory, and contractile phenotypes.[5] The resulting excess accumulation and crosslinking of the ECM eventually disrupts cellular niches and leads to a loss of structural tissue integrity and homeostatic liver cell function.[6] Ultimately, liver fibrosis can progress to liver cirrhosis, which is associated with high morbidity and mortality due to chronic liver failure and risk of developing hepatocellular carcinoma.[7]

Metabolic dysfunction-associated steatotic liver disease (MASLD; previously termed non-alcoholic fatty liver disease or NAFLD), as the hepatic manifestation of the metabolic syndrome, is now the leading etiology of chronic liver disease,[8] and therefore, the importance of metabolic pathways in driving hepatic fibrogenesis is well recognized. However, research on the link between metabolic regulation and fibrosis has traditionally been focused on hepatocytes as the main metabolically active liver cell type. Now, we are becoming increasingly aware that the metabolic state of immune and stromal cells is closely linked to their cellular functions (Figure 1). Changes in the metabolic environment, including nutrient availability, redox state, gut-derived metabolites and intestinal microbiota, sex, ethnicity, and circadian biology dictate cell function through modulation of cellular metabolism and metabolic stress responses. Similarly, changes of cell function in response to specific immune or growth factor signals require an adaptation of cellular metabolism to match their needs.[9] Manipulation of immune and stromal cell metabolism therefore represents a promising target for therapeutic interventions in liver fibrosis.[10] We will review the cell-intrinsic metabolic changes in hepatic immune cells, focusing on macrophages, lymphoid cells, and HSCs in chronic liver injury and hepatic fibrosis.

IMMUNOMETABOLISM IN CHRONIC HEPATIC INJURY

Liver macrophages

Liver macrophages are a heterogeneous immune cell population derived from myeloid precursors and fulfill essential functions in

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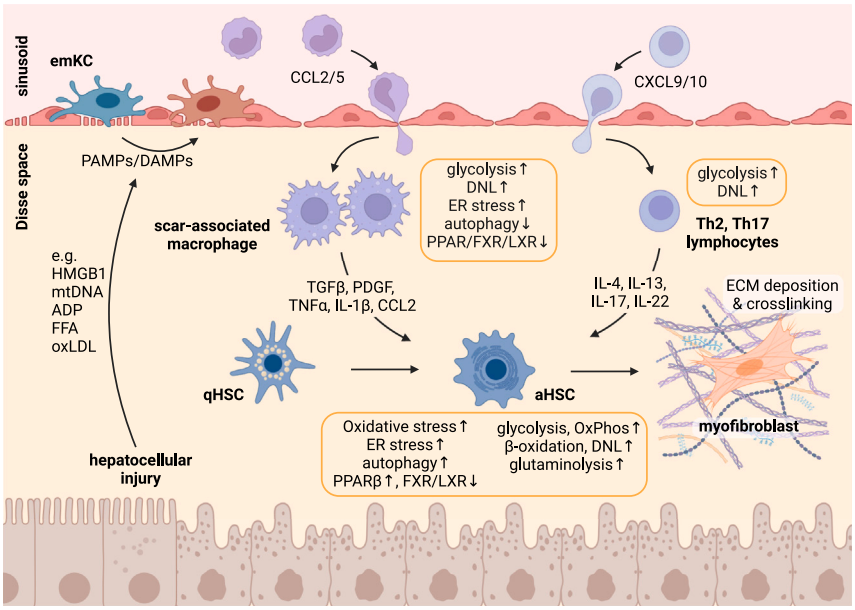

Figure 1. General mechanisms of liver fibrosis and main metabolic adaptations in macrophages, lymphocytes, and activated hepatic stellate cells

Hepatocellular injury initiates liver inflammation through the release of damage-associated signals. These signals, as well as cytokines and chemokines released by activated embryonic Kupffer cells (emKCs), recruit circulating immune cells to the liver, where they undergo pronounced phenotypical changes. Monocytes differentiate into inflammatory, scar-associated macrophages, and the adaptive immune response shifts toward Th2/17 immunity. Cytokines, growth factors, and damage-associated signals eventually drive the activation of quiescent hepatic stellate cells (qHSCs) toward an activated (aHSC), proliferative, extracellular matrix (ECM)-producing and contractile myofibroblast-like phenotype. Phenotypical changes are accompanied and dependent on pronounced metabolic adaptations, including increased glycolysis, activation of lipogenic pathways, and metabolic stress responses, including oxidative and endoplasmic reticulum stress, as well as autophagy and ferroptosis, and changes in nuclear receptor signaling.

ADP, adenosine diphosphate; CCL, C-C motif ligand; CXCL, C-X-C motif ligand; DAMPs, damage-associated molecular patterns; DNL, *de novo* lipogenesis; ER, endoplasmic reticulum; FFA, free fatty acid; FXR, farnesoid X receptor; HMGB1, high mobility group box 1; (q/a)HSC, (quiescent/activated) hepatic stellate cell; IL, interleukin; LXR, liver X receptor; MMP, matrix metalloproteinase; mtDNA, mitochondrial DNA; oxLDL, oxidized low-density lipoprotein; OxPhos, oxidative phosphorylation; PAMPs, pathogen-associated molecular patterns; STAT-3, signal transducer and activator of transcription 3; PPAR, peroxisome proliferator-activated receptor; TGF-β, transforming growth factor beta. Created with BioRender.com.

the progression and resolution of chronic hepatic injury.[11,12] The main liver-resident macrophage population under homeostatic conditions is the embryonically derived Kupffer cells, which are characterized by their high phagocytic activity and sensing capacity for damage- and pathogen-associated molecular patterns (DAMPs and PAMPs).[13] Thereby, they act as early responders to damage signals and, upon activation, promote liver inflammation through the release of proinflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor alpha (TNF-α).[14] The other main hepatic macrophage population consists of monocyte-derived macrophages, which differentiate from circulating monocytes that infiltrate the liver upon liver injury. Once infiltrated, these inflammatory monocytes (Ly-6C^{high} in mice/CD14^{+} in humans) differentiate into macrophages, which—dependent on microenvironmental cues—will readily acquire different phenotypes with diverse functions.[12] Single-cell and spatial sequencing technologies have revealed the high plasticity of this cell population and identified CD9^{+} TREM2^{+} scar-associated macrophages (SAMs) as the main modulators of fibrogenic processes in human chronic liver injury.[15] This observation is shared by murine models of liver fibrosis,[15–18] across other species as well as in different organs.[19,20] SAMs can secrete fibrogenic factors, including transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), TNF-α, and IL-1β, thereby activating HSCs to produce collagen.[5] Conversely, infiltrating monocyte-derived macrophages can take on a restorative phenotype that is characterized by high expression of anti-inflammatory mediators, angiogenic and growth factors, and matrix metalloproteinases,[21–23] as well as by high efferocytotic capacity to clear debris and dead cells and thereby promote liver fibrosis regression.[24,25]

Hepatic macrophages are highly spatially organized, and this has important implications for liver disease progression.[26–28] However, it is yet unclear how this relates to hepatic metabolic zonation or oxygen and nutrient gradients. Accumulating evidence shows that changes in macrophage function depend on pronounced adaptations in macrophage metabolism and cellular stress responses (Figure 2), and the contribution of cellular metabolism to these functional switches is currently being unraveled.

Carbohydrate metabolism and the Warburg effect

It is well established that, generally, inflammatory macrophages are characterized by increased glycolysis and tricarboxylic acid (TCA) cycle activity, whereas regenerative macrophages fulfill their metabolic demands mainly through fatty acid oxidation and activation of the arginase pathway.[29] Pyruvate kinase M2 (PKM2) has been identified as a central, targetable metabolic switch in this process, highly similar to the Warburg effect observed in malignant cell transformation in cancer.[30] Phosphorylation of PKM2 inhibits its enzymatic activity but promotes nuclear translocation and hypoxia-inducible factor (HIF)-1α-dependent activation of glycolytic transcriptional programs.[31,32] In murine models of MASLD, phosphorylation of PKM2 and subsequently increased glycolytic activity lead to a shift in macrophage polarization toward an inflammatory phenotype that promotes steatohepatitis and fibrosis.[33,34] Consequently, blocking the nuclear translocation of PKM2,[34] or inhibiting PKM2 binding factors such as the activin/bone morphogenetic protein antagonist follistatin-like 1 (FSTL1),[35] Annexin A5,[33] or the HSP70 family member HSPA12A,[36] effectively prevents the development of steatohepatitis and liver fibrosis in murine models of metabolic dysfunction-associated steatohepatitis (MASH). Excess concentrations of fructose, a putative dietary driver of steatohepatitis through its steatogenic metabolism in hepatocytes, also promote an inflammatory macrophage phenotype through increased glycolysis and oxidative phosphorylation (OxPhos),[37]

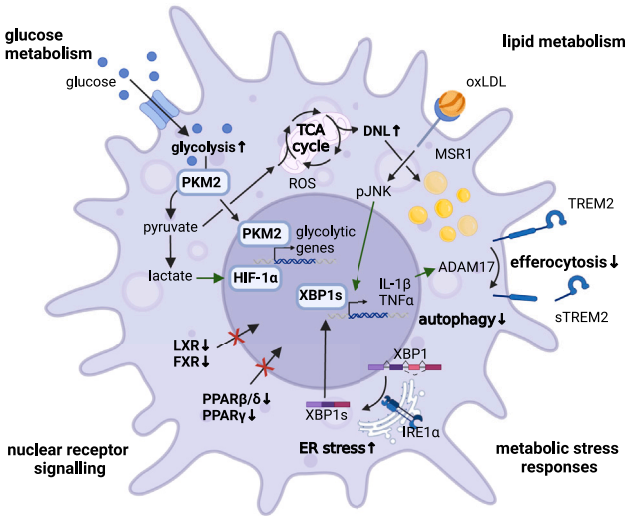

Figure 2. Metabolic reprogramming in hepatic macrophage activation during liver fibrogenesis

Macrophage activation is accompanied by pronounced adaptations in substrate metabolism and metabolic stress responses. Increased glycolysis provides energy and nuclear translocation of pyruvate kinase M2 (PKM2), and induction of HIF-1α upregulates glycolytic gene expression. Pyruvate subsequently feeds into the tricarboxylic acid (TCA) cycle and *de novo* lipogenesis (DNL), providing lipids for macrophage lipid accumulation. In addition, lipids are taken up, e.g., through macrophage scavenger receptor 1 (MSR1), which, through c-Jun N-terminal kinase (JNK) signaling, promotes inflammatory activation. In addition, macrophage activation is characterized by pronounced activation of the endoplasmic reticulum (ER) stress response, with inositol-requiring enzyme 1 alpha (IRE1α)-mediated X-box binding protein 1 (XBP1) signaling driving inflammatory activation and altered metabolic gene expression profile. Metabolism-induced macrophage inflammation drives expression of ADAM metallopeptidase domain 17 (ADAM17), which cleaves the triggering receptor expressed on myeloid cells 2 (TREM2) protein on the cell surface and licenses macrophage inflammation through disrupted efferocytosis. Inflammatory macrophages are characterized by reduced nuclear receptor signaling, including peroxisome proliferator-activated receptors (PPARs), liver X receptor (LXR), and farnesoid X receptor (FXR).

IL-1β, interleukin 1 beta; oxLDL, oxidized low-density lipoprotein; TNF-α, tumor necrosis factor alpha; ROS, reactive oxygen species. Created with BioRender.com.

though this has not yet been demonstrated in the context of liver fibrosis. Still, high glycolytic activity is not entirely specific to inflammatory macrophage activation. A recent report highlighted that the efferocytotic capacity of regenerative macrophages relies on rapid activation of glycolysis to fulfill associated high energy demands, a process dependent on the activation of the glycolytic enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2 (PFKFB2).[38] Overall, inflammatory macrophage activation in steatohepatitis is dependent on increased glycolysis, but such a metabolic switch might also be involved in alternative macrophage responses, which calls for caution when targeting this pathway therapeutically.

TCA cycle and ketone bodies

Importantly, in inflammatory macrophages, the TCA is broken after citrate and succinate and decoupled from OxPhos.[29] Consecutively accumulating succinate promotes the production of IL-1β by HIF1α signaling.[39] Furthermore, respiratory chain uncoupling by uncoupling protein 1 (UCP1) is required to clear extracellular succinate in a Western diet-induced mouse model of MASLD.[40] Conversely, the extracellular accumulation of succinate in *Ucp1* knockout mice supported inflammatory activation of macrophages and promoted liver inflammation and fibrosis, and these processes were dependent on succinate receptor 1 (SUCNR1) signaling.[40]

Ketone bodies, namely acetoacetate and β-hydroxybutyrate, are alternative energy sources provided by hepatocytes under conditions of glucose starvation. Hepatic macrophages can use acetoacetate, which, through succinyl-coenzyme A (CoA)-oxoacid transferase (SCOT), feeds into the TCA cycle.[41] Myeloid-specific knockout of *Scot* resulted in increased release of fibrogenic growth factors from macrophages, increased fibrogenic gene expression in HSCs, and subsequently aggravated liver fibrosis in a murine high-fat diet.[41] Strikingly, injection of acetoacetate was able to prevent ECM deposition in this model,[41] indicating a potential role of this pathway in liver fibrosis. In addition, macrophages can also signal back to hepatocytes; resident liver macrophages were shown to influence ketogenesis in hepatocytes during fasting, as the glucocorticoid receptor (GR) modulates the fasting-induced macrophage secretome and impacts GR/peroxisome proliferator-activated receptor α (PPARα) target gene activation in hepatocytes.[42]

Changes in lipid metabolism

Furthermore, lipid accumulation and lipotoxic stress are tightly linked to inflammatory macrophage activation, a process that is particularly relevant in steatohepatitis. Lipid overload and lipotoxicity promote differentiation of macrophages toward an inflammatory phenotype.[43] Using single-cell technologies, this specific lipid-associated macrophage (LAM) phenotype has been characterized in different lipid-associated disease conditions, including steatohepatitis,[15,19] obesity-induced adipose tissue dysfunction,[44] and atherosclerosis,[45] and is closely related to the SAM phenotype found in fibrotic livers. The high transcriptional similarity of murine and human LAM populations in steatohepatitis further highlights the relevance of this specific metabolic phenotype in human disease conditions.[19] In steatohepatitis, these LAMs accumulate around steatotic hepatocytes in so-called hepatic crown-like structures and—depending on their efferocytotic capacity—can limit inflammation by containing local danger-associated signals.[25] However, upon prolonged injury, LAMs develop a pronounced inflammatory phenotype through toxic lipid signaling, e.g., through macrophage scavenging receptor 1 (MSR1),[46] and lose their efferocytotic functions,[25] ultimately fueling the progression to fibrosis. The association between hepatocellular steatosis and macrophage activation is bidirectional, and inflammatory macrophages can inhibit beta-oxidation in steatotic hepatocytes through the secretion of TNF-α and IL-1β and thereby perpetuate lipotoxic liver injury.[47–50]

In the final step of triacylglycerol breakdown, monoacylglycerol lipase (MAGL) hydrolyses the breakdown of monoacylglycerols to acetyl-CoA and glycerol, a process that is a major source of arachidonic acid, which feeds into the generation of inflammatory lipid mediators, including prostaglandins.[51] Myeloid-specific knockout of MAGL in murine models of toxic and biliary fibrosis results in reduced inflammation and fibrosis, as well as a shift in macrophage populations from inflammatory Ly-6C^{high} to adaptive Ly-6C^{low} cells.[52] In a subsequent study, the authors showed that these MAGL-deficient macrophages display no apparent changes in typical inflammatory and anti-inflammatory

genes, such as IL-6 or IL-10, but are characterized by significantly increased expression of interferon (IFN)γ signature genes, which delayed liver regeneration in acute CCl_4-induced toxic liver injury.[53] Interestingly, the protective effects of myeloid *MAGL* knockout or pharmacological MAGL inhibition are dependent on increased autophagic activity in macrophages, demonstrated by increased inflammation and fibrosis in *MAGL* and *ATG5* double knockouts.[52] Indeed, fibrosis resolution in mice was associated with increased autophagy signaling, implicating macrophage autophagy as an important mechanism in driving fibrosis regression.[54]

Metabolic stress responses in macrophage activation

The UPR and ER stress. Endoplasmic reticulum (ER) stress represents an important link between hepatocellular injury and liver inflammation. Oxidative stress, increased abundance of free fatty acids, hyperglycemia, lipotoxicity, and the pronounced increase in protein synthesis following cell activation result in the accumulation of unfolded proteins. These are recognized by conserved sensors located in the ER, namely activating transcription factor 6 (ATF6), protein kinase RNA-like ER kinase (PERK), and inositol-requiring enzyme 1 alpha (IRE1α).[55] Activation of these sensor protein complexes results in signaling cascades that drive increased c-Jun N-terminal kinase (JNK) and nuclear factor κB (NF-κB) activity, unconventional splicing of the UPR regulator X-box-binding protein 1 (XBP1) as well as IRE1α-dependent pathways regulating digestion and inactivation of mRNAs and microRNAs (miRNAs).[55] Eventually, in response to ER stress, cells downregulate protein synthesis, upregulate their protein folding capacity, and induce lipid biosynthesis.[55] ER stress, and the IRE1α-XBP1 pathway in particular, is an important regulator in inflammatory macrophage activation.[56,57] Indeed, in several murine models of fibrosis, myeloid-specific deletion of *Xbp1* led to improved liver histology and a shift toward restorative macrophage phenotypes, mediated by reduced activation of the NOD-, LRR-, and pyrin-domain-containing protein 3 (NLRP3) inflammasome pathway.[50,58] Future studies will need to investigate the contribution of the PERK and ATF6 branches of the ER stress response to macrophage activation in liver fibrosis.

Autophagy. Autophagy is a catabolic pathway of lysosomal cellular self-digestion. It consists of different processes, namely macroautophagy through double-membrane autophagosomes; direct processing of cytoplasmic proteins or organelles in microautophagy; chaperone-mediated autophagy, which recognizes specific cytosolic proteins; and non-canonical forms of autophagy.[59] In liver macrophages, canonic autophagy dampens inflammatory responses and macrophages in liver fibrosis are characterized by reduced canonic autophagic activity.[60,61] Myeloid-specific deletion of autophagy-related gene 5 (*Atg5*), which encodes for a pivotal protein in the assembly of the autophagosome, leads to increased inflammatory macrophage activation *in vitro*, which translates into IL-1α/β-dependent activation of HSCs and exacerbated CCl_4-induced murine liver fibrosis.[60] Conversely, gadolinium-induced macrophage depletion abolished the fibrogenic effects of adenovirus-mediated *Atg5* knockdown in murine CCl_4-induced fibrosis.[62] Similarly, myeloid-specific deletion of lysosome-associated membrane protein 2A (LAMP2A), the rate-limiting protein in chaperone-mediated autophagy, exacerbated high-fat-fructose-cholesterol-induced liver injury.[61] This was mediated by reduced degradation of Nup85, which promotes monocyte recruitment and aggravates inflammatory activation.[61] The notion of reduced macrophage canonical autophagy as a driver of liver fibrosis progression is underpinned by findings of pro-resolution macrophages showing an autophagic and phagocytotic phenotype.[54] However, LC3-associated phagocytosis (LAP), a non-canonical form of autophagy, is upregulated in monocytes and macrophages in human and murine chronic injury and represents an adaptive response.[63] Inhibition of LAP aggravated CCl_4-induced liver injury and fibrosis as it reduced anti-inflammatory Fc fragment of IgG receptor IIA (FcγRIIA) signaling.[63] In summary, reduced canonical autophagy in macrophages has been established as a driver of fibrogenesis. The specific mechanisms by which macrophage autophagy drives fibrosis regression and the contribution of non-canonical pathways warrant further study.

Iron metabolism and ferroptosis. Iron accumulation can be observed in several fibrotic conditions and drives cellular senescence and activation of fibrogenic pathways.[64] In chronic liver disease, liver macrophages accumulate iron, which promotes their inflammatory activation and prevents their anti-inflammatory, alternative differentiation.[65] In MASH, macrophages associated with crown-like structures also show an increased abundance of intracellular iron, and iron chelation attenuates the inflammatory response and fibrosis area in a Western diet plus CCl_4-induced murine liver fibrosis.[66]

Cellular iron overload is closely associated with the generation of reactive oxygen species (ROS) and lipid peroxidation, which can culminate in non-apoptotic cell death called ferroptosis.[67] Although hepatocellular ferroptosis can be considered "immunogenic" and is implicated in driving immune cell recruitment, inflammatory macrophage activation, liver inflammation, and fibrosis,[68,69] the contribution of macrophage-intrinsic ferroptosis is less clear and needs further study.

Nuclear receptors in the regulation of macrophage metabolism and phenotype

Nuclear receptors serve as transcription factors regulating glucose, fat, and cholesterol metabolism, and in addition to their roles in hepatocellular metabolism, their roles in shaping immune cell functions are currently receiving particular attention.[70]

PPARs are a group of nuclear receptors and are activated through the binding of fatty acids, eicosanoids, and other fatty acid derivatives.[71] Three different types have been identified, with different cell types and organ distribution: PPARα, PPARβ/δ, and PPARγ. In macrophages, the activation of PPARα increases cholesterol efflux, and in atherosclerosis it has been shown that this reduces inflammatory macrophage activation.[72] Treatment with PPARα agonists reduces macrophage inflammatory activation and fibrosis in murine models of steatohepatitis, but this is probably mediated by effects on hepatocellular lipid metabolism,[73,74] particularly because PPARα expression in hepatic macrophages is considerably low.[75] PPARβ/δ is important for mediating the anti-inflammatory effects of IL-4 on liver-resident macrophages.[76] Similarly, hematopoietic knockout of *Ppard* results in reduced hepatic anti-inflammatory gene expression and promotes insulin resistance and steatosis.[76] PPARδ activation promotes an anti-inflammatory/restorative phenotype in hepatic macrophages,[75] yet the effects of myeloid PPARδ on

liver fibrosis are not fully understood. PPARγ regulates fatty acid and cholesterol transporter genes, as well as TCA cycle genes, thereby increasing macrophage lipid handling capacity, cholesterol efflux, and OxPhos[77] and promoting anti-inflammatory macrophage polarization.[78] Furthermore, PPARγ enables macrophage glutamine metabolism, which is required for their anti-inflammatory activation.[79] Although monocytes infiltration in acute CCl_4 injury is not reduced by either specific or pan-PPAR agonism,[75] pan-PPAR agonist treatment increases lipid-associated gene expression and blunts inflammatory activation in palmitic-acid-treated bone-marrow-derived macrophages *in vitro*.[75]

Liver X receptors (LXRs-alpha/beta, NR1H3/2) are also highly expressed in hepatic macrophages.[77] Activated mainly by oxysterols, LXRs regulate fatty acid and cholesterol transport as well as glycolytic genes, resulting in increased cholesterol efflux and reduced glycolysis.[77] Consequently, LXR activation is associated with reduced inflammatory macrophage activation,[80,81] and myeloid-specific deletion of *Nr1h2/3* promotes inflammation and atherosclerosis in $Ldlr^{-/-}$-induced hypercholesterinemia.[82] Similarly in diet-induced murine steatohepatitis, whole-body *Nr1h3* knockout leads to aggravated liver inflammation,[83] while myeloid-specific effects have not been demonstrated yet.

The nuclear bile acid receptor farnesoid X receptor (FXR, NR1H4) is expressed in Kupffer cells,[19] and *in vitro* pharmacological activation of FXR signaling shifts macrophage differentiation toward an anti-inflammatory profile.[84,85] Similarly, the synthetic bile acid and FXR agonist obeticholic acid (OCA) represses chemokine CCL2 (MCP-1) expression by Kupffer cells in response to LPS/TNF-α co-stimulation.[86] In murine sclerosing cholangitis models, myeloid-specific *Nr1h4* knockout exacerbates hepatic injury and inflammation and leads to a shift toward T helper (Th)1/Th17 immune responses.[87] Furthermore, myeloid knockout of *Nr1h4* renders systemic treatment with FXR agonists ineffective, indicating that activation of myeloid FXR mediates a significant proportion of these therapeutic effects.[87] However, how FXR impacts macrophages immunometabolism will need to be addressed in future studies.

Lymphocytes and ILCs

Lymphocytes and innate lymphoid cells (ILCs) have important roles in the adaptive immune response. In liver injury, B and T lymphocytes mediate and modulate disease activity in antigen-mediated conditions such as viral hepatitis or autoimmune liver diseases. However, they are also involved in other types of liver injury, as non-specific activation of the adaptive immune system may be directed against injured hepatocytes.[3] Liver fibrosis is characterized by a shift toward Th2 and Th17 helper cell responses, resulting in the release of corresponding cytokines, particularly IL-4, IL-13, IL-17, and IL-22, which facilitate HSC activation and ECM secretion.[88]

There is little direct evidence on how metabolic changes impact the differentiation of hepatic lymphocytes and ILCs. Still, in general, activation and proliferation of lymphocytes and ILCs is associated with a shift of cellular metabolism to aerobic glycolysis, lipogenesis, and amino acid metabolism (arginine, glutamine, serine, and glycine),[89] again providing cells with energy and building blocks for protein synthesis to enable their immune functions. As principally observed in mice, metabolic cues present in MASLD, such as acetate and extracellular ATP, promote the development of auto-aggressive $CD8^+$ $PD1^+$ granzyme$^+$ $FOXO1^{low}$ T cells that attack steatotic hepatocytes in a process involving purinergic signaling and, independent of major histocompatibility complex (MHC) class 1, ultimately promoting inflammation and fibrosis.[90]

Differential regulation of fatty acid oxidation and *de novo* lipogenesis (DNL) have a particularly important role in the differentiation of T cell subsets. $CD8^+$ effector T cells favor lipogenic pathways while $CD8^+$ memory T cells rely on fatty acid oxidation.[91] Similarly, in $CD4^+$ T cells, the development of Th1, Th2, and Th17 cells depends on glycolysis and fatty acid synthesis, whereas regulatory T cell (Treg) differentiation requires increased fatty acid oxidation and is characterized by lower rates of glycolysis.[91] Central regulators in this metabolic switch are the Myc-mTORC1-sterol regulatory-element-binding protein (SREBP)-pathway, which induces lipogenesis through the upregulation of acetyl-CoA carboxylase (ACC), and activation of AMP-activated protein kinase (AMPK), which stimulates fatty acid oxidation through suppression of mTORC1 activity, PPAR-γ coactivator (PGC)-1 activation, and subsequent upregulation of CPT1.[91]

In liver injury, PKM2 again seems to play a vital role in the metabolic shift of T cells. It has been demonstrated that inflammatory activation of hepatic Th17 in MASLD is dependent on increased glycolytic activity, mediated through PKM2 activation.[92] Genetic deletion of PKM2 alleviated liver injury and reduced macrophage numbers in a murine high-fat diet, though effects on liver fibrosis had not been investigated.[92] Regarding lipogenesis, ACC inhibition prevented liver fibrosis in a choline-deficient dietary MASH model in mice and significantly reduced primary human T cell differentiation into Th17 cells.[93] Furthermore, circulating Treg in cirrhosis are characterized by Nrf2 signaling, which mediates mitochondrial dysfunction and decreases fatty acid oxidation rate.[94] However, whether modulating lymphocyte and ILC metabolism affects the progression of liver fibrosis still needs to be investigated.

METABOLIC REPROGRAMMING IN HSC ACTIVATION

HSCs are the main fibrogenic cell population of the liver. Under homeostasis, quiescent HSCs serve as retinol-storing pericytes of the liver and regulate sinusoidal blood flow.[95] Upon liver injury, however, a plethora of damage-associated, inflammatory, and metabolic signals lead to HSC activation, loss of retinol droplets, and transdifferentiation into a myofibroblast-like phenotype. This phenotype is associated with increased proliferation, contractility, migration, and inflammatory signaling as well as increased expression of ECM components, which ultimately results in the aberrant deposition of ECM, with subsequent development of fibrosis and cirrhosis.[5] The highly energy-demanding phenotypic switch toward a myofibroblast-like phenotype requires substantial metabolic reprogramming characterized by increased glycolysis, lipid droplet mobilization, beta oxidation, and glutaminolysis, as well as activation of cell-intrinsic stress pathways such as the unfolded protein response (UPR) and ER stress (Figure 3).

Carbohydrate metabolism and OxPhos

Similar to the Warburg effect in cancer cells and to the metabolic switch in hepatic inflammatory immune cells (outlined above),

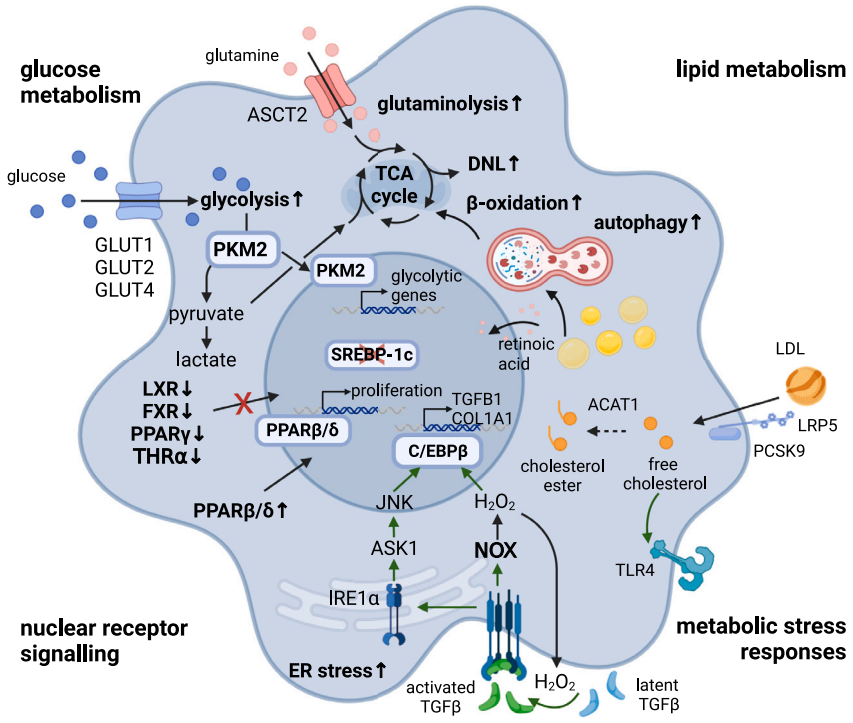

Figure 3. Metabolic reprogramming in fibrogenic hepatic stellate cell activation

Upon activation, hepatic stellate cells (HSCs) undergo a pronounced phenotypic change that involves increased substrate availability to provide energy and building blocks. Activated HSCs obtain their energy through increased glycolysis, tricarboxylic acid (TCA) cycle activity, and mobilization of stored lipids that feed into beta oxidation, and nuclear translocation of pyruvate kinase M2 (PKM2) regulates metabolic gene expression. Glutamine represents an alternative source and enters the cell through alanine/serine/cysteine/threonine transporter 1 (ASCT1) before being metabolized to glutamate and α-ketoglutarate to feed into the TCA cycle. Furthermore, HSCs upregulate autophagic pathways to generate energy from cell organelles, including retinol-storing lipid droplets. Activated HSCs are abundant in free cholesterol, partly taken up through low-density lipoprotein receptor-related protein 5 (LRP5) interacting with proprotein convertase subtilisin/kexin type 9 (PCSK9), and cholesterol promotes expression of Toll-like receptor 4 (TLR4) and sensitizes HSCs to transforming growth factor beta (TGF-β) signaling. In addition, increased abundance of unfolded proteins and TGF-β signaling elicits an endoplasmic reticulum (ER) stress response through inositol-requiring enzyme 1 alpha (IRE1α), which upregulates fibrogenic gene expression through apoptosis-signal-regulating kinase 1 (ASK1)/c-Jun N-terminal kinase (JNK) signaling. In a self-perpetuating cycle, TGF-β signaling induces NADPH oxidase activity to produce H_2O_2, which supports fibrogenic gene expression and enables activation and release of extracellular latent TGF-β. Like macrophages, HSC activation is characterized by reduced nuclear receptor signaling (e.g. LXR, FXR, or THRα), although peroxisome proliferator-activated receptor β/δ is upregulated in activated HSCs and promotes their proliferation.
ACAT1, acetyl-CoA acetyltransferase; DNL, *de novo* lipogenesis; FXR, farnesoid X receptor; GLUT, glucose transporter; LXR, liver X receptor; LDL, low-density lipoprotein; SREBP-1c, sterol regulatory-element-binding protein 1; THR, thyroid hormone receptor. Created with BioRender.com.

activated HSCs increase their glycolytic activity with upregulation of glucose transporters (e.g., GLUT1) and rate-limiting glycolytic enzymes, including hexokinase-2, PKM2, and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3), and inhibition of glycolysis reverted the activated HSC phenotype.[96] Similar changes in HSC carbohydrate metabolism have been observed in CCl_4- and bile duct ligation-induced murine and rat models of liver fibrosis.[96,97] Oroxylin A, a flavonoid with diverse biological functions, inhibits glycolysis and fibrogenic activity of HSCs *in vitro* and reduced CCl_4-induced murine liver fibrosis.[98] These effects were dependent on lactate dehydrogenase-A (LDH-A), demonstrated by adenoviral overexpression of LDH-A that abrogated glycolytic effects *in vivo* and *in vitro*.[98] Similar to processes outlined for metabolic reprogramming in liver macrophages, the metabolic switch in HSCs is dependent on nuclear translocation of PKM2, whose inhibition can alleviate HSC activation and fibrosis.[99] Furthermore, HSC activation depends on increased activity of the rate-limiting glycolytic enzyme hexokinase-2, a process mediated by increased lactate production and subsequent histone lactylation *in vitro*.[100] Notably, HSC-specific knockout of hexokinase-2 almost completely prevented CCl_4-induced murine liver fibrosis.[100] Interestingly, exosomes released from activated HSC also contain GLUT1 and PKM2, and their delivery to other non-parenchymal cells induced glycolysis in HSCs, Kupffer cells, and sinusoidal endothelial cells *in vitro*.[101]

Liver fibrosis and HSC activation are also highly dependent on mitochondrial biogenesis and increased rates of OxPhos. In liver fibrosis, activated HSCs are characterized by higher mitochondria numbers and increased mitochondrial respiratory capacity.[102] Activation of the human HSC line LX-2 or primary rat HSCs led to an increased abundance of mitochondria and increased oxygen consumption rate, as well as extracellular acidification rates, reflecting increased OxPhos.[103] Promoting mitochondrial fission through *Fis1* overexpression leads to HSC activation and increased OxPhos *in vitro*.[104] Conversely, inhibition of mitochondrial fission in CCl_4-treated mice significantly alleviated liver fibrosis.[104] Notably, glycolysis and OxPhos have differential effects on *in vitro* HSC activation: while the inhibition of glycolysis had no effect on rat HSC proliferation but reduced *Acta2* expression, the inhibition of OxPhos reduced proliferative activity and suppressed fibrogenic gene expression.[105]

The mitochondrial function in HSCs is also influenced by genetic factors. For instance, the *PNPLA3* rs738409 C>G gene variant (encoding for I148M), a well-known risk factor for fibrosis in MASLD, reduces antioxidant capacity in human HSCs due to respiratory chain complex IV insufficiency, while ROS secretion and fibrogenic TGF-β signaling are increased.[106]

Lipid metabolism

Quiescent HSCs are characterized by storage of vitamin-A-rich lipid droplets, and the presence of vitamin A and insulin signaling are required for maintaining the quiescent phenotype.[107] Upon activation, HSCs quickly lose their lipid droplets and undergo sustained changes in their lipid metabolism, a process largely driven by loss of expression of the adipogenic transcription

factors CCAAT/enhancer-binding protein (C/EBP) α, C/EBPβ, C/EBPδ, PPARγ, LXRα, and SREBP 1c.[108]

Inhibition of DNL, e.g., by blocking ACC or fatty acid synthetase (FASN), is currently being tested as a therapeutic approach in steatotic liver diseases (SLDs) and can lead to reductions in liver fibrosis in murine models of SLD.[93] Strikingly, these effects are not exclusively mediated by improved hepatocellular metabolism. HSCs also show increased activity of lipogenic pathways.[109] Blocking DNL in HSCs by inhibiting FASN or ACC1/2 effectively reduced fibrogenic gene expression in rodent and human primary HSCs *in vitro*,[93,109,110] and this was associated with decreased glycolytic flux and OxPhos.[109] However, the relevance of lipogenic pathways in non-metabolic-mediated liver fibrosis has not been demonstrated.

Stearoyl-CoA desaturase (SCD) is the rate-limiting enzyme in the synthesis of mono-unsaturated fatty acids. SCD expression is increased in human and murine liver fibrosis, and in activated HSCs, and promotes fibrosis and hepatocellular carcinogenesis.[111]

Only recently has it been demonstrated that activated HSCs are characterized by increased activity of beta-oxidation.[112] In human liver fibrosis as well as murine models of CCl_4- and metabolically induced liver fibrosis, HSCs strikingly upregulate carnitine palmitoyl transferase 1A (CPT1A), the rate-limiting enzyme in beta oxidation of medium- and long-chain fatty acids.[112] Genetic silencing or pharmacological inhibition of CPT1A *in vitro* as well as HSC-specific genetic knockout of *Cpt1a* in murine choline-deficient models of liver fibrosis were able to reduce fibrogenic HSC activation and liver fibrosis.[112]

In MASLD, free cholesterol accumulates in HSCs, contributing to fibrogenic HSC activation.[113] However, cholesterol can contribute to fibrogenic HSC activation in other types of liver injury as well: in murine CCl_4- and BDL-induced liver fibrosis, a high-cholesterol diet aggravates liver fibrosis.[114] Strikingly, this effect seems independent of liver inflammation and liver macrophage activation, as demonstrated by a persistent pro-fibrotic effect after clodronate-induced macrophage depletion.[114] Mechanistically, free cholesterol accumulates in HSCs, supported by a disruption in the SREBP2 negative feedback loop, which leads to increased TLR4 expression and sensitizes HSCs to TGF-β-induced activation.[113] As HSC activation is mediated by free cholesterol but not cholesterol esters, some have investigated the role of acyl-CoA:cholesterol acyltransferase 1 (ACAT1), the enzyme catalyzing the conversion from free cholesterol to cholesterol ester. HSC-specific knockout of *Acat1* severely aggravated free-cholesterol-induced effects on HSCs *in vitro* and promoted murine CCl_4- and BDL-induced liver fibrosis.[115] This process was dependent on the upregulation of TLR4, demonstrated by abrogation of adverse *Acat1* knockout effects in conditional *Tlr4* knockout mice.[115] In HSCs, low-density lipoprotein (LDL) cholesterol uptake is partly mediated by LDL-related protein 5 (LRP5), dependent on its complex formation with proprotein convertase subtilisin/kexin 9 (PCSK9).[116] Whether this route of cholesterol uptake contributes to HSC activation still needs to be investigated.

In the context of MASH, where spatially distinct interactions between lipid-laden hepatocytes and inflammatory foamy macrophages initiate and propagate fibrosis, these metabolic activation circuits in HSCs may play a major role. Indeed, a recent report showed that fibrosis may be initiated in the area surrounding these lipid- and cholesterol-rich hepatic crown-like structures.[117] However, the exact mechanisms and cellular interactions driving HSC activation in the context of these hepatic crown-like structures remain to be defined.

Glutaminolysis

Activated HSCs partly satisfy their energy demands through glutaminolysis,[118] a process where glutamine is metabolized to α-ketoglutarate, which feeds into the citrate cycle to generate ATP. Increased circulating glutamate/glutamine ratio in individuals with MASH correlates with liver fibrosis.[119] Mediated through hedgehog signaling, activated HSCs upregulate glutaminase, the rate-limiting enzyme catalyzing the hydrolytic deamination of glutamine to glutamate, and pharmacologic inhibition of glutaminase prevents CCl_4-induced murine liver fibrosis.[118] Recently, it has been demonstrated that glutaminolysis in HSCs can also be modulated by targeting the amino acid transporter alanine serine cysteine transporter 2 (ASCT2). Genetic knockdown of *ASCT2* in the human HSC line LX-2 reduced the abundance of glutamate and α-ketoglutarate, thereby limiting the oxygen consumption rate and HSC activation.[120] In murine chronic CCl_4-induced liver fibrosis, *Asct2* knockdown significantly reduced HSC activation and the collagen-positive area while overexpression substantially aggravated this process,[120] and pharmacologic inhibition of ASCT2 had similar anti-fibrotic effects.[121] Of note, HSCs can interact with hepatocytes through glutamate signaling. HSCs sense glutamate released from hepatocytes in alcohol-related liver disease (ALD) through glutamate receptor 5 (GluR5), and thus activated HSCs promote hepatocellular lipogenesis through the release of the endocannabinoid ligand 2-arachidonoylglycerol (2-AG).[122] However, GluR5 expression and 2-AG release tapered down during fibrogenic activation of HSCs, and therefore, a direct contribution of this process to liver fibrosis seems unlikely.

Metabolic stress responses in HSCs

The activation of HSCs is accompanied by a sustained cellular stress response, including increased oxidative stress, activation of the UPR, subsequent ER stress, and autophagy. Here, we want to give a general overview of the principal mechanisms and highlight some novel developments. More detailed accounts are available elsewhere.[95]

Oxidative stress

Activated HSCs are characterized by increased oxidative stress, which plays an important role in fibrogenic activation and collagen production. Several classical activators of HSCs, including TGF-β and PDGF, induce the expression of NADPH oxidase (NOX) enzymes, which is mediated by activation of the MAPK/ERK pathway.[123–125] Subsequently, NOX enzymes generate H_2O_2, which is involved in hepatic fibrogenesis in several ways: H_2O_2 contributes to an altered redox state, with an increased abundance of ROS, that is required for the activation of latent TGF-β[126]; furthermore, H_2O_2 promotes binding of the transcription factor C/EBPβ to its binding sites in the type I collagen and TGF-β1 promotor, thereby mediating collagen production and increased expression of fibrogenic TGF-β1.[127,128] Consequently, inhibition or genetic deletion of NOX enzymes attenuates fibrotic responses in HSCs and blunts fibrosis

development in murine models of liver disease.[129–131] Similarly, a recent report showed that iron overload in HSCs in MASH drives fibrogenic HSC activation through induction of ROS production, which was abrogated by anti-oxidative treatment with N-acetylcysteine.[132]

The UPR and ER stress

The induction of UPR and ER stress is an early event in HSC activation and murine liver fibrosis. However, the UPR and the ER stress pathway alone are not sufficient to activate HSCs.[133] Still, both are required for adequate HSC activation, as demonstrated by reduced fibrogenic responses in HSCs overexpressing the chaperone glucose-regulated protein 78.[134] Furthermore, TGF-β1 signaling can directly induce IRE1α phosphorylation, which, through ASK1 and JNK phosphorylation, promotes activation of the TGF-β1 downstream effector transcription factor C/EBPβ in LX-2 cells.[135] Similarly, the IRE1α-XBP1 pathway mediates TGF-β-induced upregulation of transport and Golgi organization 1 (TANGO1), which promotes type I collagen secretion from HSCs.[136]

Autophagy, ferroptosis, and senescence

In HSCs, activation of the ER stress pathway is tightly linked to the induction of autophagy, mediated through the IRE1α-XBP1s branch.[137,138] Furthermore, macrophages can promote HSC autophagy. This has been shown for LAMs that produce prostaglandin E2 (PGE2), which induces HSC autophagy through its receptor EP4.[139] Autophagy in HSCs results in the mobilization of lipid droplets, which is associated with loss of stellate cell retinol storage and increased beta oxidation rate,[140,141] indicating that the increase in autophagy serves to provide energy for HSC activation. Accordingly, HSC-specific genetic deletion of *Atg5* or *Atg7* resulted in resistance to fibrogenic activation and reduced fibrosis in CCl_4- and TAA-induced murine liver fibrosis,[141] and inhibition of autophagy through blocking the PGE2 receptor EP4 reduced methionine-choline deficiency-induced liver fibrosis.[139] Furthermore, autophagy is the major mediator of ER-stress-induced HSC activation, as deletion of *Atg7* abrogates the effects of IRE1α-XBP1 signaling.[137]

In HSCs, ferroptosis is closely linked to autophagy, and small interfering RNA (siRNA)-mediated knockdown of the autophagy regulator Beclin-1 (*Becn1*) reduced the activation of ferroptosis in HSC culture models.[142] Interestingly, stellate cell ferroptosis appears to play an anti-fibrotic role. HSC-specific knockdown of *Elavl1*, a transcript required for HSC ferroptosis, as well as treatment with the ferroptosis inducers sorafenib or the anti-malaria drug dihydroartemisinin (DHA), alleviate bile duct ligation- or CCl_4-induced murine liver fibrosis *in vivo*.[142–144] Notably, induction of ferroptosis with sorafenib was not observed in macrophages and hepatocytes and thus appears to be specific to HSCs.[143] However, chronic iron overload induces ferroptosis in both hepatocytes and stellate cells and still promotes hepatic fibrogenesis.[145]

Cellular senescence of HSCs, a condition of cell-cycle arrest during which the cells remain metabolically activated, has been observed during liver fibrosis progression and was initially linked to HSC deactivation.[146] However, in advanced stages of fibrosis, HSC senescence appears to be an important source of inflammatory and fibrogenic signals through the senescence-associated secretory phenotype (SASP), which promotes inflammation and a pro-tumoral hepatic microenvironment.[147] Using advanced single-cell analyses, senescent HSCs that are marked by expression of urokinase plasminogen activated receptor (uPAR) were found to arise from activated HSCs in murine and human MASLD-related fibrosis.[148] Targeting of senescence by uPAR-specific, senolytic chimeric antigen receptor (CAR) T cells has been demonstrated in mouse models of liver fibrosis,[149] although this may not be entirely specific to senescent HSCs. By contrast, cell-type-specific induction of senescence in HSCs by conditional deletion of yes-associated protein (*Yap1*) in established liver injury alleviated liver fibrosis in both biliary and diet-induced mouse models of advanced liver fibrosis,[150] in line with earlier findings that pharmacological inhibition of YAP attenuates CCl_4-induced murine liver fibrosis.[151] Still, others have found opposite effects of YAP inhibition in ischemia-reperfusion injury, where it delayed liver regeneration and exacerbated liver fibrosis.[152] Consequently, the exact role of HSC senescence in liver fibrosis progression needs to be better defined before developing specific therapeutic approaches.

Nuclear receptors

The role of nuclear receptors in HSC activation is less well understood, which may be related to their overall lower expression in this cell type. HSCs express PPARβ but no PPARα or PPARγ.[153] PPARβ expression is upregulated in HSC activation and promotes HSC proliferation *in vitro* and in murine CCl_4-induced liver fibrosis *in vivo*.[153,154]

Conversely, LXR ligands suppress HSC activation *in vitro*, which translates into increased susceptibility to methionine choline deficiency and CCl_4-induced murine liver injury in *Nr1h2/3* knockout mice.[155] The liver injury phenotype in *Nr1h2/3* knockout mice was unchanged by bone marrow transplantation from wild-type animals, indicating that effects are indeed mediated by stellate cells.[155] Mechanistically, LXR deficiency alters lipid handling and retinoid metabolism in HSCs, resulting in larger lipid droplets and increased capacity to mobilize and respond to retinoic acid, overall shifting HSCs toward a more activated cell state.[156]

FXR/NR1H4 is expressed in HSC and hepatic myofibroblasts to a lesser extent.[157] Still, endogenous FXR ligands dampen HSC fibrogenic response, dependent on the presence of small heterodimer partner (SHP).[158] However, another study found no effects on HSC activation when treating rodent or human HSC models with the synthetic bile acid and FXR agonist OCA.[86] Still, quiescent HSCs are enriched for FXR signaling, which is lost upon fibrogenic activation.[159] Although whole-body *Nr1h4* knockout increases the HSC activation state, OCA was again without effect.[159] Therefore, HSC-targeted modulation of FXR activity will need to demonstrate whether this bile acid receptor has a role in HSC activation.

Thyroid hormones (THs) transmit their cellular responses mainly through activation of the nuclear receptors TH receptor α and/or β (THRα/β). While THs mediate their (beneficial) metabolic actions in hepatocytes via THRβ, THRα represents the dominant isoform in human and murine HSCs.[160] Functionally, THRα represses the fibrogenic activation and TGF-β signaling in HSC.[161]

THERAPEUTIC PERSPECTIVES

Liver inflammation and fibrotic responses are intricately linked to the metabolic state of the cells involved. No compound directly

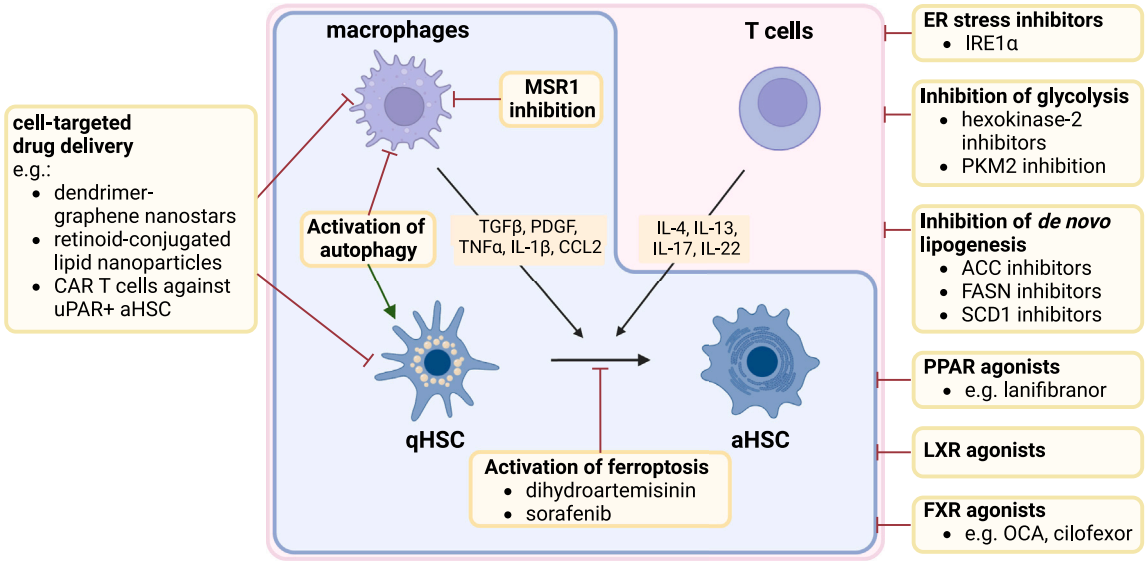

Figure 4. Immunometabolic therapeutic targets in liver fibrosis

Targeting metabolic regulation in hepatic immune and stromal cells offers novel opportunities for treating liver fibrosis. Endoplasmic reticulum (ER) stress, glycolysis, and *de novo* lipogenesis are regulated in a similar fashion in macrophages, T cells, and activated HSCs (aHSCs) and, therefore, could target the activation of all three lineages (red outline). Activation of nuclear receptor signaling through peroxisome proliferator-activated receptor (PPAR), liver X receptor (LXR), or farnesoid X receptor (FXR) elicits anti-inflammatory and anti-fibrotic effects on macrophages and HSCs (blue outline), though effects on hepatic lymphoid populations have not yet been described. Furthermore, autophagy and ferroptosis have discrete roles in macrophage and stellate cell activation. Although autophagy is anti-inflammatory in macrophages, it is critically involved in the activation of HSCs. Similarly, ferroptosis has anti-fibrotic effects and limits HSC activation, while overall its net effect is understood to be more proinflammatory. In addition, at least in metabolic-dysfunction-associated steatohepatitis, targeting macrophage scavenging receptor 1 (MSR1) may limit lipid-induced macrophage inflammation. Not least, considering the complex interplay of metabolic pathways, cell-targeted drug delivery may help in reducing off-target effects.

ACC, acetyl-CoA carboxylase; CCL2, C-C-motif ligand 2; ER, endoplasmic reticulum; FASN, fatty acid synthetase; FFA, free fatty acid; IL, interleukin; IRE1α, inositol-requiring enzyme 1 alpha; MSR1, macrophage scavenging receptor 1; OCA, obeticholic acid; PKM2, pyruvate kinase M2; PDGF, platelet-derived growth factor; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; SCD-1, stearoyl-CoA desaturase; TNF-α, tumor necrosis factor alpha. Created with BioRender.com.

targeting inflammatory or fibrogenic pathways has received regulatory approval for treating liver disease yet, and a number of drugs, including the apoptosis-signal-regulating kinase 1 (ASK1) antagonist selonsertib[162] and the dual CCR2/5 antagonist cenicriviroc,[163] have failed to reach primary endpoints (related to fibrosis) in phase 3 clinical trials. This likely reflects the complexity of the underlying disease processes, where hepatocellular injury, inflammation, and fibrogenic cell activation are closely interlinked.[164] Therefore, single pathways downstream in the disease cascade may be highly redundant and inducing a cessation of injury is likely paramount for therapeutic success. This is demonstrated by the high efficacy of substantial weight loss following bariatric surgery, which reached the primary endpoint of MASH resolution without worsening of fibrosis at 1-year follow-up in 56% of participants compared with only 16% in the lifestyle intervention arm.[165] The overlap of metabolic responses to hepatic injury in different cell types therefore represents an opportunity for targeting multiple cell types at once and upstream in the disease cascade (Figure 4).

Increased glycolysis and activation of lipogenic pathways is a hallmark of inflammatory and fibrogenic activation of macrophages, lymphocytes, and HSCs, mirroring hepatocellular metabolic changes associated with MASLD.[9] Therefore, targeting these pathways may be a viable approach in targeting liver fibrosis progression, and several compounds are already in preclinical or clinical development. Blocking rate-limiting glycolytic enzymes such as hexokinase isoforms is a straightforward approach and has shown anti-fibrotic efficacy in animal models. PF-06835919, an inhibitor of ketohexokinase, the rate-limiting enzyme in fructose metabolism, is currently in clinical testing for treating MASLD.[166] Accumulating evidence suggests that PKM2, also a rate-limiting glycolytic enzyme and a central regulator inducing Warburg effect, is a promising target. The allosteric PKM2 activator TEPP-46, which stabilizes its tetrameric complexes and thereby prevents nuclear translocation, has shown promising effects on liver inflammation and fibrosis *in vitro*.[99] In addition, several compounds targeting lipogenic pathways are currently in clinical development. Pharmacological inhibition of ACC1 was effective in reducing liver inflammation and fibrogenic gene expression in murine MASH models[167] and liver steatosis in a human phase 2a study.[168] Inhibition of SCD1 has also been tested in the clinical setting on individuals with MASH and non-significantly reduced liver fat, with no clear signal on liver inflammation or fibrosis.[169] Still another promising approach may be pharmacological activation of the cellular energy sensor, AMP-activated protein kinase (AMPK), which blocks anabolic pathways such as lipogenesis[170] and may also have a beneficial cardiovascular profile.[171] Several phytotherapeutics have been tested in MASH that exert their anti-inflammatory and anti-fibrotic effects via AMPK activation,[172,173] and small molecular compounds are currently in development.[174]

Metabolic stress responses, including ER stress and the UPR, autophagy, and ferroptosis represent alternative therapeutic targets. Blocking IRE1α and its downstream effector caspase-2 has consistently shown protective effects on liver steatosis and inflammation in animal models.[50,55,175] Still, transient and low-grade activation of the ER stress pathway is an adaptive response in obesity,[176] and complete hepatocellular blockade exacerbates liver injury,[177,178] which needs to be considered when developing pharmacological treatments. The diverging roles of autophagy and ferroptosis in different cell types, as outlined for macrophages and HSCs, may limit their applicability as therapeutic targets, and warrant further investigation. However, whole-body knockout of *Atg7* exacerbates insulin resistance and ER stress,[179] while overexpression of *Atg5* extends lifespan[180] and pharmacological approaches activating autophagy have shown protective effects from liver fibrosis,[181] indicating a net beneficial effect of autophagy.

Activation of nuclear receptors is arguably the most developed approach to targeting immunometabolism in liver fibrosis. Evidence suggests that the effects of FXR agonists are largely mediated through action on myeloid cells.[87] The FXR agonist OCA is approved as a second-line treatment in primary biliary cholangitis; OCA and other FXR agonists have been investigated as a treatment in MASLD fibrosis.[10] However, the development of OCA as an anti-fibrotic therapy in MASH has been terminated because the FDA did not provide accelerated approval based on available efficacy and safety data for this compound.[182] PPAR agonists, particularly those targeting PPARβ/δ, are well characterized for their efficacy in promoting anti-inflammatory macrophage polarization,[70,71,75] and the pan-PPAR agonist lanifibranor is in late clinical development, with promising clinical data in a phase 2b study.[183] Similarly, LXR activity is associated with anti-inflammatory macrophage activation[82] and suppresses HSC activation.[156] Notably, the THRβ agonist resmetirom has recently received accelerated approval by the FDA for the treatment of MASH fibrosis, following positive data from the registrational phase 3 MAESTRO-NASH trial.[184] However, as mentioned above, THRα is the predominant THR isoform in HSCs[161] and innate immune cells, including macrophages.[185] Therefore, the anti-inflammatory and anti-fibrotic effects of resmetirom are mainly mediated through hepatocellular metabolic adaptations and likely not by direct immunometabolic effects.

When targeting metabolic pathways, we should be particularly wary of side effects because drug actions will not be restricted to the targeted tissue. Prominent examples are PPAR agonists, which are potent at inducing anti-inflammatory macrophage phenotypes and limiting HSC activation, but their clinical use is currently restricted due to side effects such as cardiovascular events, osteopenia, edema, and fluid retention.[71] Novel methods of drug delivery could enable cell-type-specific targeting of metabolic pathways and thereby potentially prevent unwanted off-target effects. For example, macrophage-specific delivery of the non-thiazolidinedione PPARγ agonist GW1929 by dendrimer-graphene nanostars in murine CCl_4-induced liver injury was able to shift macrophage phenotypes toward an anti-inflammatory, alternative phenotype and effectively prevented fibrosis development.[186]

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Hepatic fibrogenesis is dependent on metabolic reprogramming of all liver cell populations. Overall, fibrogenic activation of macrophages, lymphocytes, and HSCs is characterized by a Warburg-like effect, with increased rates of glycolysis and lipogenesis, and evidence supports the notion that the targeting of metabolic pathways is a promising approach for halting and reversing liver fibrosis. Still, considering the heterogeneity and complexity of immune and stroma cells, this view is likely oversimplified and future research will need to address the metabolic adaptations that support the differentiation of distinct phenotypes. We know that the classification of macrophages into alternatively activated, inflammatory, or pro-resolution states, as well as HSCs into quiescent, activated, and inactivated states, is oversimplified. This notion is particularly important considering evidence that macrophage phenotypes in the resolution of injury share some metabolic programs with those that are driving inflammatory processes.[38] Therefore, detailed knowledge of the metabolic processes that accompany these differentiation trajectories will be paramount to develop targeted therapies.

This review provided a cell-centric view on hepatic metabolic processes and how these are shaping pro-fibrotic cell states. Importantly, metabolic and functional cell states are largely governed by intercellular crosstalk involving all hepatic cells, including hepatocytes, biliary epithelial cells, endothelial cells, and myeloid and lymphoid immune cells, as well as the mesenchymal compartment.[187] This cellular crosstalk is enhanced by spatial proximity of cells, where cell-cell contacts, cell-matrix interactions, and paracrine and autocrine signaling circuits provide specific functional niches that determine cell phenotypes. Our understanding of cellular interactions and the development of pro-fibrotic cell niches in chronic liver disease, and the consequences of therapeutic targeting, has significantly expanded in recent years and is extensively reviewed elsewhere.[10,164,187] However, how cell-intrinsic metabolic regulation, particularly in non-parenchymal cells, feeds into these circuits largely remains to be determined.

Integration of emerging and state-of-the-art methods, including single-cell and spatial (multi-)metabolomics technologies,[188] advanced intravital imaging, and preclinical animal and *in vitro* models will bring us unbiased and multidimensional insights into the cellular processes, cell-cell communication networks, and how these relate to metabolic cell states and disruption of homeostatic cell niches.[187] It appears realistic to expect that this will provide us with a more holistic understanding of the mechanisms driving liver fibrosis progression to enable diagnostic and therapeutic advances.

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